

学校编码: 10384

分类号\_\_密级\_\_

学号: 20520091151323

UDC\_\_

厦 门 大 学

硕 士 学 位 论 文

一个评价海水中重金属氧化胁迫效应的新指标

A New Index to Evaluate the Oxidative Stress Degree from  
Metals in Seawater

徐春来

指导教师姓名: 杨利民 副教授

专 业 名 称: 分 析 化 学

论文提交日期: 2013 年 7 月

论文答辩时间: 2013 年 7 月

学位授予日期: 2013 年 月

答辩委员会主席: \_\_\_\_\_

评 阅 人: \_\_\_\_\_

2013 年 7 月

## 厦门大学学位论文原创性声明

本人呈交的学位论文是本人在导师指导下,独立完成的研究成果。本人在论文写作中参考其他个人或集体已经发表的研究成果,均在文中以适当方式明确标明,并符合法律规范和《厦门大学研究生学术活动规范(试行)》。

另外,该学位论文为( )课题(组)的研究成果,获得( )课题(组)经费或实验室的资助,在( )实验室完成。(请在以上括号内填写课题或课题组负责人或实验室名称,未有此项声明内容的,可以不作特别声明。)

声明人(签名):

201 年 月 日

# 厦门大学学位论文著作权使用声明

本人同意厦门大学根据《中华人民共和国学位条例暂行实施办法》等规定保留和使用此学位论文，并向主管部门或其指定机构送交学位论文（包括纸质版和电子版），允许学位论文进入厦门大学图书馆及其数据库被查阅、借阅。本人同意厦门大学将学位论文加入全国博士、硕士学位论文共建单位数据库进行检索，将学位论文的标题和摘要汇编出版，采用影印、缩印或者其它方式合理复制学位论文。

本学位论文属于：

（        ） 1. 经厦门大学保密委员会审查核定的保密学位论文，  
于        年        月        日解密，解密后适用上述授权。

（        ） 2. 不保密，适用上述授权。

（请在以上相应括号内打“√”或填上相应内容。保密学位论文应是已经厦门大学保密委员会审定过的学位论文，未经厦门大学保密委员会审定的学位论文均为公开学位论文。此声明栏不填写的，默认为公开学位论文，均适用上述授权。）

声明人（签名）：

201        年        月

# 目录

摘要.....	i
Abstract.....	iv
第一章 前言 .....	1
1.1 水体重金属污染.....	1
1.1.1 水体重金属污染的现状.....	1
1.1.2 水体重金属污染的危害.....	2
1.1.3 水体重金属污染的治理.....	3
1.2 水中重金属对藻类的毒性作用 .....	4
1.2.1 重金属对藻类生长、繁殖的影响.....	4
1.2.2 重金属对藻类生理、生化功能的影响.....	5
1.3 藻类对重金属的耐受机理.....	6
1.3.1 细胞壁的钝化作用.....	6
1.3.2 细胞外排物.....	7
1.3.3 谷胱甘肽 (GSH) 和植物螯合肽 Phytochelatin (PCs)等低分子量含巯基化合物.....	7
1.3.4 植物金属硫蛋白 Metallothioneins (MT) .....	10
1.3.5 有机酸和氨基酸.....	12
1.3.6 抗氧化保护系统.....	12
1.3.7 胁迫蛋白.....	13
1.4 巯基的定量分析进展 .....	14
1.4.1 UV .....	14
1.4.2 FL.....	15
1.4.2.1 马来酰亚胺类 .....	15
1.4.2.2 碘乙酰胺类 (-NHCOCH <sub>2</sub> I) .....	16
1.4.2.3 活性卤素类 .....	17

1.4.3 ECD.....	17
1.4.4 分子质谱.....	18
1.4.5 ICP-MS .....	18
1.4.6 AFS .....	19
1.5 选题依据和研究内容.....	20
1.6 参考文献.....	21
第二章 Cd、Cu、Pb 胁迫对三角褐指藻的生理毒性及藻体内合成的含巯基低分子量化合物的种类 .....	33
2.1 前言.....	33
2.2 实验部分.....	34
2.2.1 仪器及试剂.....	34
2.2.1.1 实验仪器 .....	34
2.2.1.2 试剂及溶液配制 .....	35
2.2.2 实验方法.....	36
2.2.2.1 三角褐指藻及其培养条件 .....	36
2.2.2.2 三角褐指藻在 Cd、Cu、Pb 胁迫下的生长情况及在细胞内外的分布 .....	36
2.2.2.3 三角褐指藻细胞内含 Cd 多肽/蛋白的提取和筛选 .....	37
2.2.2.4 植物螯合肽(Phytochelatins, PCs)及其他含巯基小分子的提取、种类鉴别.....	38
2.3 结果与讨论.....	38
2.3.1 三角褐指藻在 Cu、Cd 和 Pb 胁迫下的生长状况 .....	38
2.3.2 三角褐指藻对 Cu、Cd 和 Pb 的吸收规律 .....	42
2.3.3 三角褐指藻细胞内含金属多肽/蛋白的筛选 .....	46
2.3.4 三角褐指藻在 Cu、Cd、Pb 胁迫下产生的小分子量化合物的种类.....	52
2.3.4.1 三角褐指藻在不同金属胁迫条件下产生的植物螯合肽 .....	52
2.3.4.2 植物螯合肽的质谱鉴定 .....	55

2.4 结论 .....	57
2.5 参考文献 .....	58
第三章 还原型巯基和氧化型巯基含量测定及比值变化机制研究 .....	60
3.1 前言 .....	60
3.2 实验部分 .....	61
3.2.1 仪器及试剂 .....	61
3.2.1.1 实验仪器 .....	61
3.2.1.2 试剂及溶液的配制 .....	61
3.2.2 实验方法 .....	62
3.2.2.1 三角褐指藻及其培养条件 .....	62
3.2.2.2 微小原甲藻( <i>Prorocentrum minimum</i> )和伪矮海链藻( <i>Thalassiosira pseudonana</i> )及其培养条件 .....	63
3.2.2.3 植物螯合肽(Phytochelatins, PCs)及其他含巯基小分子不同型态定量分析 .....	63
3.3 结果与讨论 .....	64
3.3.1 色谱条件的优化 .....	64
3.3.2 HPLC-UV 和 HPLC-(UV/TiO <sub>2</sub> PCRD)-AFS 的定量结果的比较 .....	65
3.3.3 不同胁迫条件下三角褐指藻体内还原型巯基含量和巯基总含量测定及 $R_{Total}^{Red}$ 和 $R_{Oxd}^{Red}$ 的准确值 .....	67
3.3.4 不同胁迫条件下三角褐指藻体内 $R_{Total}^{Red}$ 和 $R_{Oxd}^{Red}$ 的变化机理。 .....	76
3.4 结论 .....	81
3.5 参考文献 .....	81
第四章 总结与展望 .....	84
4.1 总结 .....	84
4.2 展望 .....	85

在校期间发表的论文 .....	86
-----------------	----

致 谢 .....	87
-----------	----

厦门大学博士论文摘要库

## Contents

<b>Abstract(in Chinese)</b> .....	<b>i</b>
<b>Abstract (in English)</b> .....	<b>iv</b>
<b>Chapter 1 Preface</b> .....	<b>1</b>
<b>1.1 Heavy metal pollution of waters</b> .....	<b>1</b>
1.1.1 Pollution status of heavy metals in waters .....	1
1.1.2 Hazards of heavy metal pollution in waters.....	2
1.1.3 Remediation of heavy metal-contaminated waters .....	3
<b>1.2 Toxicity effect of heavy metal on algae</b> .....	<b>4</b>
1.2.1 The effect of heavy metal on the growth and propagation of algae.....	4
1.2.2 The effect of heavy metal on the biochemical and physiological of algae .....	5
<b>1.3 Cellular mechanism for heavy metal tolerance in algae</b> .....	<b>6</b>
1.3.1 The binding property of cell wall.....	6
1.3.2 The exudates released by algae .....	7
1.3.3 Low molecular weight compounds like glutathione(GSH) and phytochelatins(PCs) .....	7
1.3.4 Metallothioneins.....	10
1.3.5 Organic acids and amino acids .....	12
1.3.6 System of antioxidation .....	12
1.3.7 Stress proteins .....	13
<b>1.4 Quantification analysis of sulfhydryl</b> .....	<b>14</b>
1.4.1 UV .....	14
1.4.2 FL.....	15
1.4.2.1 Maleimide.....	15



1.4.2.2 Iodoacetamide (-NHCOCH <sub>2</sub> I) .....	16
1.4.2.3 Halides .....	17
1.4.3 ECD.....	17
1.4.4 ESI-MS .....	18
1.4.5 ICP-MS .....	18
1.4.6 AFS .....	19
<b>1.5 The basis and content of this study .....</b>	<b>20</b>
<b>1.6 References .....</b>	<b>21</b>
 <b>Chapter 2 The ecotoxicity of Cd,Cu,and Pb to <i>Phaeodactylum tricornutum</i> and low molecular weight compounds that containing sulfhydryl synthesized in algae cells .....</b>	 <b>33</b>
<b>2.1 Introduction .....</b>	<b>33</b>
<b>2.2 Experimental.....</b>	<b>34</b>
2.2.1 Instruments and reagents .....	34
2.2.1.1 Instruments .....	34
2.2.1.2 Reagents and solutions preparation .....	35
2.2.2 Methods.....	36
2.2.2.1 <i>P.tricornutum</i> and Cultivation .....	36
2.2.2.2 Growth of <i>P. tricornutum</i> under Cd,Cu and Pb stress and the content of intracellular and extracellular metal .....	36
2.2.2.3 Screening of metal binding peptides and ptoteins in the cell of <i>P. tricornutum</i> .....	37
2.2.2.4 Extraction and identification of low molecular weight compounds that containing sulfydryl group like phytochelatins .....	38
<b>2.3 Results and discussion.....</b>	<b>38</b>
2.3.1 Growth of <i>P. tricornutum</i> under Cu,Cd and Pb stress.....	38
2.3.2 Rule of Cu,Cd and Pb uptake by <i>P. tricornutum</i> .....	42
2.3.3 Screening of metal binding peptides and ptoteins in the cell of <i>P.</i>	

<i>tricornutum</i> .....	46
2.3.4 Identification of low molecular weight compounds that containing sulfhydryl group in <i>P.tricornutum</i> .....	52
2.3.4.1 Analysis of phytochelatin produced by <i>P. tricornutum</i> .....	52
2.3.4.2 The structure identification by ESI-MS .....	55
<b>2.4 Conclusions</b> .....	<b>57</b>
<b>2.5 References</b> .....	<b>58</b>
<b>Chapter 3 The quantification of reduced form and oxidized form sulfhydryl and the mechnism of their ratio change</b> .....	<b>60</b>
<b>3.1 Introduction</b> .....	<b>60</b>
<b>3.2 Experimental</b> .....	<b>61</b>
3.2.1 Instrument and reagents .....	61
3.2.1.1 Instruments .....	61
3.2.1.2 Reagents and solution preparation .....	61
3.2.2 Methods.....	62
3.2.2.1 <i>P.tricornutum</i> and Cultivation .....	62
3.2.2.2 <i>Prorocentrum minimum</i> , <i>Thalassiosira pseudonana</i> and Cultivation....	63
3.2.2.3 Quantification of reduced and oxidized form sulfhydryl of low molecular weight compounds like phytochelatins(PCs) .....	63
<b>3.3 Results and discussion</b> .....	<b>64</b>
3.3.1 Seperation of PHMB labeled Thiols .....	64
3.3.2 The quantification results of HPLC-UV and HPLC-(UV/TiO <sub>2</sub> PCRD)-AFS .....	65
3.3.3 The quantification of reduced form and tatal sulfhydryl and the accurate value of $R_{Total}^{Red}$ and $R_{Oxd}^{Red}$ .....	67
3.3.4 The mechanism of the change of $R_{Total}^{Red}$ and $R_{Oxd}^{Red}$ under different stress conditions .....	76

<b>3.4 Conclusions .....</b>	<b>81</b>
<b>3.5 References .....</b>	<b>81</b>
<b>Chapter 4 Summary and Prospects.....</b>	<b>84</b>
<b>4.1 Summary .....</b>	<b>84</b>
<b>4.2 Prospects.....</b>	<b>85</b>
<b>Publications.....</b>	<b>86</b>
<b>Acknowledgement.....</b>	<b>87</b>

## 摘要

重金属的毒性和其在环境中的迁移转化给环境中的生物体造成毒害作用。相对于在海水中重金属的总浓度,因为海水中有各种无机和有机配体的存在在一定程度上改变了重金属元素的存在状态,其可被生物吸收和利用的浓度更加重要。氧化胁迫是重金属离子对于生物体产生毒害的主要途径之一。尽管已有很多生物标志物被用来指示环境中金属离子对生物体的氧化胁迫程度,但是这些标志物并不是很灵敏,特别是在生物体受到金属离子短期胁迫时,已有的标志物无法快速指示氧化胁迫的程度;即使在表征长期胁迫时,为了得到可靠的结果,往往需同时对其中多个标志物进行测定。因此,更为灵敏且准确的氧化胁迫指示方法亟待发展。本研究以海洋中广泛存在的三角褐指藻为藻类的模型,研究  $\text{CuCl}_2$ 、 $\text{CdCl}_2$  和  $\text{PbCl}_2$  对藻的长期和短期氧化胁迫;以期在分子水平上获得更多关于金属离子对于生物体的氧化胁迫程度的信息,发现有效的反映生物体受重金属氧化胁迫程度的生物分子并发展新的指示生物体受氧化胁迫的程度的指标和方法。同时获取环境中生物可利用金属元素存在状态的信息。

第一章主要简要地介绍了水体重金属污染的现状和水体治理的必要性以及藻类耐受重金属污染的生物化学机制的研究进展,并对含巯基化合物分析技术进行了简要介绍,最后提出了我们的选题依据。

第二章主要研究了在不同浓度(0、0.05、0.5、1、10 和 20  $\mu\text{M}$ ) 和不同胁迫时间(6 小时和 3 天)条件下,  $\text{CuCl}_2$ 、 $\text{CdCl}_2$  和  $\text{PbCl}_2$  对三角褐指藻的胁迫和毒理作用。我们监测了在不同胁迫条件下,藻的生长情况,发现经过 3 天的胁迫,与空白组比较,胁迫后三角褐指藻藻细胞的密度整体下降。在金属胁迫浓度较高时,  $\text{Cu}$  表现出最强的毒性,  $\text{Pb}$  表现出最弱的毒性,  $\text{Cd}$  的毒性介于两者之间。例如,当胁迫浓度为 20  $\mu\text{M}$  时,胁迫时间为 3 天,和空白相比(未加金属离子),  $\text{Cu}$  胁迫的藻细胞密度下降 51.4%,  $\text{Pb}$  胁迫的藻细胞密度下降 26.1%,  $\text{Cd}$  胁迫的下降 39.7%。与此同时,我们还通过 ICP-MS 检测了不同胁迫条件下细胞内部和细胞外的金属元素含量。发现当胁迫浓度和胁迫时间增大时,细胞内部和细胞外吸附的金属量都在增大。细胞外吸附的金属量要明显大于细胞内的金属量。例如,

当 20  $\mu\text{M}$   $\text{CdCl}_2$  胁迫三角褐指藻 3 天时, 细胞外吸附的 Cd 为 908 amol per cell, 细胞内吸附的量为 275 amol per cell; 当  $\text{PbCl}_2$  胁迫三角褐指藻 3 天, 胁迫浓度从 0.05 增大到 20  $\mu\text{M}$  时, 细胞内的 Pb 从 29.4 amol per cell 增大到 334 amol per cell。我们通过 SEC-ICP-MS 考查了金属和细胞内物质结合的情况。我们发现 Cu 和 Cd 主要是和细胞内大分子量化合物 (MW > 20 kDa) 结合在一起, Pb 主要和分子量较小的化合物 (MW < 1500 Da) 结合在一起。最后我们通过 HPLC-ESI-MS 来鉴定胁迫过程中产生的含巯基 (-SH) 小分子量化合物的种类。我们发现这类化合物主要有半胱氨酸 (Cys)、谷胱甘肽 (GSH) 和植物螯合肽 (PCs, 主要是  $\text{PC}_{2-6}$ )。

第三章主要开展了重金属对藻类生物的氧化还原胁迫程度研究。提出利用前文所述的藻细胞内含巯基小分子化合物的还原形态占总量的比例 ( $R_{\text{Total}}^{\text{Red}}$  = 还原型巯基的浓度/巯基的总浓度) 或与氧化形态的比值 ( $R_{\text{Oxd}}^{\text{Red}}$  = 还原型巯基的浓度/氧化型巯基的浓度) 来指示藻细胞受氧化还原胁迫的程度。含巯基小分子化合物的还原 (-SH) 和氧化 (-S-S-) 状态含量的比值不仅是反映藻细胞所处环境的氧化还原程度, 而且在对抗重金属的氧化胁迫过程中藻细胞中 -SH 和 -S-S- 的转化也起到了非常重要的调节作用。为了准确得到还原型巯基小分的量, 我们用  $\text{NaOCOC}_6\text{H}_4\text{HgOH}$  (PHMB) 来标记和稳定 -SH。所标记 Hg 可以通过 UV/ $\text{TiO}_2$  光催化还原进行原子化, 进而实现原子荧光光谱检测, 最终通过所测得的 Hg 的量推算出 -SH 的含量。不同金属元素胁迫时,  $R_{\text{Oxd}}^{\text{Red}}$  和  $R_{\text{Total}}^{\text{Red}}$  的变化趋势不一样。相对于正常值偏离的程度, 即  $R_{\text{Oxd}}^{\text{Red}}$  和  $R_{\text{Total}}^{\text{Red}}$  的大小直接和藻细胞受到氧化胁迫的程度相关。因为不管是去除氧化活性高的物质 (可能导致  $R_{\text{Oxd}}^{\text{Red}}$  过低) 还是直接络合金属 (可能导致  $R_{\text{Oxd}}^{\text{Red}}$  过高) 都是藻细胞用来对抗环境中金属离子造成的氧化胁迫的策略。除了三角褐指藻外, 为了验证我们提出的  $R_{\text{Oxd}}^{\text{Red}}$  和  $R_{\text{Total}}^{\text{Red}}$ , 我们用了另外两种藻, 伪矮海链藻 (*Thalassiosira pseudonana*) 和微小原甲藻 (*Prorocentrum minimum*) 做平行实验, 研究它们在对照和 10  $\mu\text{M}$   $\text{CdCl}_2$  胁迫时,  $R_{\text{Total}}^{\text{Red}}$  和  $R_{\text{Oxd}}^{\text{Red}}$  值的变化。结果对于 *T. pseudonana*,  $R_{\text{Oxd}}^{\text{Red}}$  值从 1.94 (来自对照实验的对照值) 上升到 2.85; 对于 *P. minimum*,  $R_{\text{Oxd}}^{\text{Red}}$  值从 2.87 (空白值) 上升到 8.09。

同时  $\text{CdCl}_2$  胁迫过程中也伴随着藻细胞密度的明显下降。因此证明了我们所提出的  $R_{Total}^{Red}$  和  $R_{Oxd}^{Red}$  值可以作为评价生物体受金属氧化胁迫程度的指标。第四章总结了本硕士论文的研究工作，对其不足进行了讨论，并对将来进一步的研究工作进行了展望。

**关键词：** 三角褐指藻 植物螯合肽 PHMB HPLC-AFS 氧化胁迫指标

## Abstract

The toxicity of heavy metals and their translocation in the environment caused a lot of risks to the organisms living in the environment. Compared with their total concentrations in seawater, their bioavailable concentrations are more important because there are many organic and inorganic ligands in seawater. Oxidative stress is one of most important mechanisms of heavy metals towards the organisms. Many biological molecules have been used to evaluate the oxidative stress caused by the chronic and acute exposure of metal ions in the environment, but they are somewhat not sensitive especially for acute exposure of metal ions, and one has to determine many of them at the same time to get reliable results. A more sensitive index thus needs to be developed. In this thesis, *Phaeodactylum tricornutum* was selected as a model to investigate oxidative stress caused by the exposure of  $\text{CuCl}_2$ ,  $\text{CdCl}_2$  and/or  $\text{PbCl}_2$ . We aimed to get more information at molecular level about oxidative stress caused by metal ions towards the organisms in seawater, and to develop more sensitive indexes to evaluate the oxidative stress caused by metal ions, and to obtain information regarding the bioavailable metal ions that may exist in the environment.

In Chapter One, the state-of-the art for controlling heavy metal pollution in waters were introduced. A variety of potential mechanisms that may be involved in algae's detoxification and tolerance to heavy metals at the molecular level were introduced, and the analytical techniques for analyzing sulfhydryl group(-SH) containing compounds were also introduced as well. Based on the brief review, my research proposal was thus made for this thesis.

In Chapter two, *P. tricornutum* was cultured under the stress of the different concentrations (0, 0.05, 0.5, 1, 10, 20  $\mu\text{M}$ ) of  $\text{CuCl}_2$ ,  $\text{CdCl}_2$  and/or  $\text{PbCl}_2$  for 6 hour and/or 3 day. We monitored the growth rate of *P. tricornutum* under different stressed conditions finding that after 3d exposure of the metal ions the cellular density of *P. tricornutum* generally decreased as the increase of the metal concentrations. For

example, when the stressed metal concentration was 20  $\mu\text{M}$ , after 3 d exposure, compared with that of the control experiments, the cellular density decreased 51.4% for  $\text{CuCl}_2$ , 26.1% for  $\text{PbCl}_2$  and 39.7% for  $\text{CdCl}_2$ , indicating that Cu is most toxic one, and followed the order of  $\text{Cu}^{2+} > \text{Cd}^{2+} > \text{Pb}^{2+}$  under the exposure of this high concentration of metal ions. In the meantime, the intracellular and extracellular metal content was determined using ICP-MS. We found that the intracellular and extracellular metal content generally became higher as the increase of the exposure time and the concentration of metal ions. The extracellular metal content was much higher than the intracellular metal content. For example, under the stress of 20  $\mu\text{M}$   $\text{CdCl}_2$  for 3 d, the extracellular Cd was about 908 amol per cell, and the intracellular metal was about 275 amol per cell; under the stress of 0.05 to 20  $\mu\text{M}$   $\text{PbCl}_2$  for 3 d, the intracellular content of Pb arose from 29.4 to 334 amol per cell. Information regarding the distribution of the metals and their binding states with intracellular substances were studied using SEC-ICP-MS. We found that Cd and Cu mainly bound with high molecular weight substances ( $\text{MW} > 20 \text{ kDa}$ ), and Pb mainly bound with low molecular weight ligands ( $\text{MW} < 1500 \text{ Da}$ ). The species of low molecular weight compounds that containing sulfhydryl ( $-\text{SH}$ ) were further indentified using HPLC-ESI-MS, suggesting that they are cysteine (Cys), glutathione (GSH) and phytochelatins (PCs, mainly  $\text{PC}_{2-6}$ ) in *P. tricornutum* cells under stressed conditions.

In Chapter Three, the low molecular weight compounds that containing  $-\text{SH}$  mentioned above are important not only to homestat redox stress, but also the interchange between their reduced form ( $-\text{SH}$ ) and oxidized form ( $-\text{S-S}-$ ) can indicate the degree of the oxidative stress. We thus proposed that that the ratios ( $R_{\text{Total}}^{\text{Red}}$ ) of the content of reduced form thiols to the total thiols) and  $R_{\text{Oxd}}^{\text{Red}}$  (the content of reduced form thiols to the content of oxidized form thiols) might be used as indexes to evaluate oxidative stress caused by metal ions. In order to get accurate content of  $-\text{SH}$ ,  $\text{NaOCOC}_6\text{H}_4\text{HgOH}$  (PHMB) was used to stabilize and label  $-\text{SH}$ . UV/ $\text{TiO}_2$  photocatalysis reaction device (UV/ $\text{TiO}_2$  PCRd) was used as an effective sample introduction unit and an interface for mercury determination by atomic fluorescence



Degree papers are in the "[Xiamen University Electronic Theses and Dissertations Database](#)". Full texts are available in the following ways:

1. If your library is a CALIS member libraries, please log on <http://etd.calis.edu.cn/> and submit requests online, or consult the interlibrary loan department in your library.
2. For users of non-CALIS member libraries, please mail to [etd@xmu.edu.cn](mailto:etd@xmu.edu.cn) for delivery details.

厦门大学博硕士论文摘要库